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## EDITORIAL

# Spinal GABA<sub>A</sub> receptors for pain control: back to the future?

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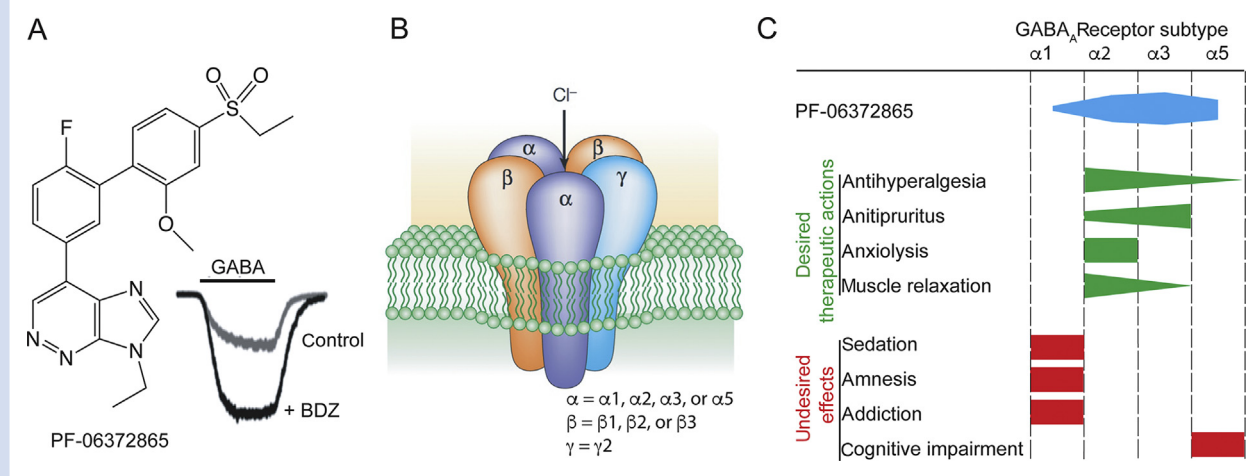
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$\gamma$ -Aminobutyric acid type A (GABA<sub>A</sub>) receptors, ligand-gated ion channel receptors for the inhibitory neurotransmitter GABA, mediate neuronal inhibition in the CNS, including the spinal cord. Based on their subunit compositions (more specifically the  $\alpha$  subunit isoform included in the pentameric receptor complex), they can be subdivided into six major subtypes (from  $\alpha 1$  to  $\alpha 6$ ). GABA<sub>A</sub> receptors containing  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ , or  $\alpha 5$  subunits are sensitive to modulation by classical benzodiazepines, whereas those containing  $\alpha 4$  or  $\alpha 6$  subunits instead of  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ , or  $\alpha 5$  subunits are insensitive. The compound PF-06372865, the subject of a study by Nickolls and colleagues<sup>1</sup> in a recent issue of the *British Journal of Anaesthesia*, was developed by Pfizer Inc (Cambridge, UK) as a partial agonist at the benzodiazepine binding sites of receptors containing  $\alpha 2$ ,  $\alpha 3$ , or  $\alpha 5$  subunits.<sup>1</sup> It is mainly this lack of activity at  $\alpha 1$  GABA<sub>A</sub> receptors that distinguishes PF-06372865 from classical benzodiazepines. Why is this important, and why should avoiding activity at  $\alpha 1$  GABA<sub>A</sub> receptors convey analgesic efficacy to benzodiazepine site ligands?

Let us first consider the neurophysiological basis of GABAergic analgesia. This concept is rooted in the gate control theory of pain, in which Melzack and Wall<sup>2</sup> proposed that inhibitory (mainly GABAergic) neurones of the spinal dorsal horn gate incoming nociceptive input and prevent the activation of 'pain' signalling projections by non-painful sensory input. A large body of evidence indicates that the efficacy of this inhibitory control is compromised in chronic pain states through several mechanisms.<sup>3</sup> Accordingly, drugs that facilitate GABAergic inhibition should in principle be able to correct this deficit. Indeed, local spinal administration of drugs that

enhance synaptic inhibition reverse heightened pathological pain sensitivity.<sup>4,5</sup> Work in mice expressing genetically engineered GABA<sub>A</sub> receptor  $\alpha$  subunits has shown that  $\alpha 2$  (and  $\alpha 3$ ) GABA<sub>A</sub> receptors are the most relevant GABA<sub>A</sub> receptor subtypes for spinal analgesia, and that  $\alpha 1$  and  $\alpha 5$  receptors contribute either nothing ( $\alpha 1$ ) or very little ( $\alpha 5$ ) to this process.<sup>5,6</sup> This profile fits well with the enriched expression of  $\alpha 2$  and  $\alpha 3$  GABA<sub>A</sub> receptors within the spinal dorsal horn, particularly the superficial layers where incoming nociceptive fibres terminate.<sup>7</sup> The dispensability of  $\alpha 1$  GABA<sub>A</sub> receptors for analgesia was a crucial finding, as receptors of this subtype cause the great majority of the unwanted effects of classical benzodiazepine agonists including sedation, memory impairment, tolerance (loss of efficacy during chronic treatment), and addiction<sup>8</sup> (Fig. 1). This segregation provides a hint as to why sparing activity at  $\alpha 1$  receptors would confer analgesic activity.

The clinically tolerated doses of benzodiazepines (for indications other than anaesthesia) are limited by undesired sedation. In fact, typical clinically used doses of classical benzodiazepines yield receptor occupancies <30%.<sup>9–12</sup> Preclinical work has shown that this is too low to yield significant relevant analgesia, explaining why classical non-selective benzodiazepines are basically devoid of analgesic efficacy. Only when activity at  $\alpha 1$  GABA<sub>A</sub> receptors is sufficiently reduced can the higher doses needed for analgesic efficacy be reached without putting a patient (or an animal) to sleep (see the work of Zeilhofer and colleagues<sup>13</sup>). Inspired by these results, several groups have tested subtype-selective benzodiazepine site agonists in various rodent pain models. Originally, these



**Fig 1.** (a) Chemical structure of PF-06372865, a subtype-selective partial agonist at the benzodiazepine-binding site of  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 5$   $\gamma$ -aminobutyric acid A (GABA<sub>A</sub>) receptors. The inset illustrates chloride currents through GABA<sub>A</sub> receptor channels recorded in the absence (control) and presence of a benzodiazepine (BDZ). (b) Schematic illustration and subunit composition of heteropentameric GABA<sub>A</sub> receptors. (c) Comparison of the subtype-selectivity of PF-06372865 (blue) with the contribution of the different GABA<sub>A</sub> receptor subtypes to desired (green) and undesired (red) *in vivo* actions of benzodiazepines.

compounds were developed by groups working in pharma companies, and in academia in the quest for non-sedative anxiolytics. The major outcome of these studies was that such compounds reverse pathological hyperalgesia in most neuropathic and inflammatory pain models and also in postoperative pain, provided that the compounds possessed sufficiently high modulatory activity and were used at sufficiently high doses. Such antihyperalgesic activity does not occur with classical (non-selective) benzodiazepines given systemically. In light of these encouraging preclinical data, scientists have eagerly awaited clinical studies.

The first clinical study of potential analgesic actions of a subtype-selective benzodiazepine site ligand (PF-06372865, the same compound studied by Nickolls and colleagues<sup>1</sup>) did not assess efficacy in experimental human pain, as expected, but rather was a phase II clinical trial in patients with chronic low back pain.<sup>14</sup> This trial yielded clearly negative results. We speculated<sup>15</sup> about the possible reason(s) for this failure such as species differences in target receptor expression and function between rodents and humans, the low predictive value of preclinical read-outs (both very fashionable critiques nowadays), an inappropriate patient population (patients with a neuropathic pain component were actually excluded), and insufficient drug dosing.

The new study provides new insights and helps to further narrow the reasons of the failed low back pain trial. PF-06372865 was tested in a battery of experimental pain models in healthy volunteers, and its efficacy was compared with that of pregabalin, the current gold standard in neuropathic pain treatment. PF-06372865 proved efficacious in several models with effect sizes comparable with those of pregabalin. Notably, these positive results suggest that selective  $\alpha 2$  and  $\alpha 3$  GABA<sub>A</sub> receptor modulators exert analgesia not only in rodents but also in man, thereby largely ruling out species differences or low predictive values of read-outs in

rodent experiments as underlying causes of the failure in the low back pain trial. Instead, the new study supports that insufficient drug dosing was the main problem in the low back pain trial. Nickolls and colleagues<sup>1</sup> tested single doses of 15 and 65 mg that led to estimated receptor occupancy of 50% and 80%, respectively, whereas the low back pain trial used smaller doses of 2.5 and 7.5 mg given twice per day, with estimated peak steady-state  $\alpha 2$  GABA<sub>A</sub> receptor occupancies of 30% and 50%.<sup>14</sup> Preclinical studies in mice indicated that ~70% of  $\alpha 2$  GABA<sub>A</sub> receptors need to be drug-bound (even when a high intrinsic activity ligand is used) to achieve a significant reduction in pain thresholds.<sup>6</sup> Therefore, the new study supports a lack of sufficient drug dosing as the most probable reason for failure of PF-06372865 in the low back pain trial. This is further supported by another recently published clinical trial on PF-06372865 that used the same dose regimens as the low back pain trial and failed to demonstrate efficacy against generalised anxiety disorders.<sup>16</sup>

Although the results of Nickolls and colleagues<sup>1</sup> generally support previous preclinical findings in mouse pain models, there are also interesting discrepancies. The most obvious in our opinion is that PF-06372865 showed efficacy against acute nociceptive pain, specifically in the mechanical pressure pain, cold pressor pain, and electrically evoked pain tests in humans. No significant efficacy was detected in acute models of hyperalgesia (the sunburn model) and against heat-evoked pain. PF-06372865 was therefore mostly effective against pain modalities that appeared resistant to GABA<sub>A</sub> receptor modulation in mouse experiments.<sup>17</sup> The reasons for these differences are not entirely clear. However, it is tempting to speculate that differences in the read-out measures are relevant. In the human study, PF-06372865 mainly affected pain tolerance thresholds but less so pain detection thresholds, which were the main readouts in the mouse pain models. Taking these differences into account, the study results are consistent

with what could have been predicted from previous preclinical experiments.

Although the new study is far from providing a definitive answer to the ongoing question of translatability in drug research and development, this was never its intended purpose. Importantly, it puts the previous negative result of the low back pain trial into perspective. If insufficient drug dosing was the reason for the failure of the low back pain trial, the good news is that clinical studies published so far have reported excellent tolerability<sup>1,14,16</sup> and suggest that there is significant space for dose augmentation before unwanted effects become dose limiting.

In the film *Back to the Future*, the main character Marty McFly successfully returns home from accidentally being sent 30 yr into the past to find his family in much better circumstances than before he left. This concept, rooted in science fiction, holds some allure in the real-life world of drug research and development. If only we could go back in time and tweak our experimental designs. The film ends with their car converted into a hovercraft flying at the camera and the words ‘To be continued...’ flashing on the screen. We hope that this is also the case for drugs targeting specific GABA<sub>A</sub> receptor subtypes in chronic pain.

### Authors' contributions

All authors have contributed equally and approve this editorial.

### Declarations of interest

The authors have no conflicts to declare.

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